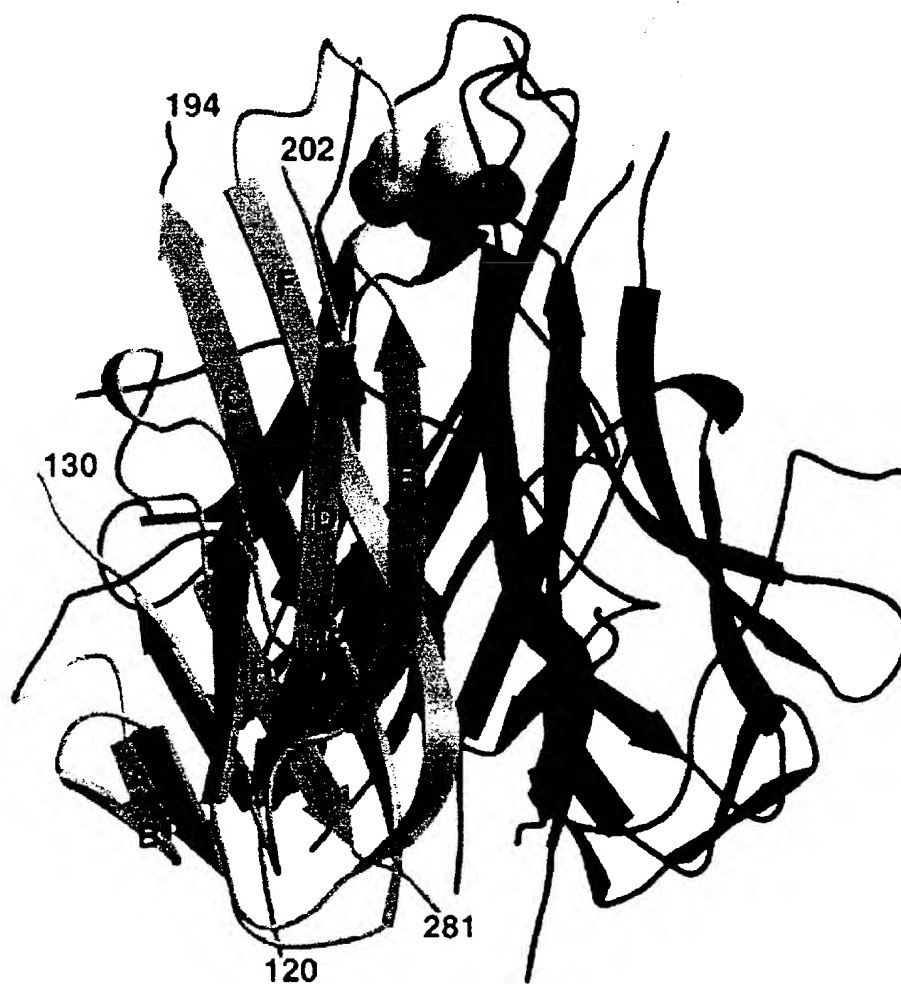


1 TTTCCCTCACTATAAAGAATAGAGAAAGGAGGCTTCAGTGACCGGCTGCCCTGGCTGACTTACAGCAGTCAGACTCTGACAGGATC  
1 ATGGCTATGATGGAGGTCACAGGGGGGACCCAGCCTGGGACAGACCTGCGTGCTGATCGTGATCTTACACAGTGCTCCTGCAGTCTCTCTGT  
1 MetAlaMetMetGluValGlnGlyGlyProSerLeuGlnThrCysValLeuIleValIlePheThrValLeuLeuGlnSerLeuCys  
181 GTGGCTGTAACTTACGTGTACTTTACCAACGAGCTGAAGCAGATGCAGGACAAGTACTCCAAAAGTGCGCATTTGCTTGTCTTAAAGAA  
31 ValAlaValThrTyrValTyrPheThrAsnGluLeuLysGlnMetGlnAspLysTyrSerLysSerGlyIleAlaCysPheLeuLysGlu  
271 GATGACAGTTATTGGGACCCCAATGACGAAGAGAGTATGAACAGCCCTGCTGGCAAGTCAAGTGGCAACTCCGTCAGCTCGTTAGAAAG  
61 AspAspSerTyrTrpAspProAsnAspGluGluSerMetAsnSerProCysTrpGlnValLysTrpGlnLeuArgGlnLeuValArgLys  
361 ATGATTTTGCAGAACCTCTGAGGAAACCATTTCTACAGTTCAAGAAAGCAACAAAATATTTCTCCCTTAGTGAGAGAAAGAGGTCCNCAG  
91 MetIleLeuArgThrSerGluGluThrIleSerThrValGlnGluLysGlnGlnAsnIleSerProLeuValArgGluArgGlyProGln  
451 AGAGTAGCAGCTCACATAACTGGGACCCAGAGGAAGAACACACATTGTCTTCTCCAAACTCCAAAGAAATGAAAAGGCTCTGGGCGCCGCAAA  
121 ArgValAlaAlaHisIleThrGlyThrArgGlyArgSerAsnThrLeuSerSerProAsnSerLysAsnGluLysAlaLeuGlyArgLys  
541 ATAAACTCCTGGGAATCATCAAGGAGTGGGCATTTCATTCCTGAGCAACTTGCACCTTGAGGAATGGTGAACCTCCATCCATGAAAAAGGG  
151 IleAsnSerTrpGluSerSerArgSerGlyHisSerPheLeuSerAsnLeuHisLeuArgAsnGlyGluLeuValIleHisGluLysGly  
631 TTTTACTACATCTATTTCCCAACACATACCTTTCGATTTTCAGGAGGAATAAAGAAAACACAAAGAACGACAAACAAATGGTCCCAATATATT  
181 PheTyrTyrIleTyrSerGlnThrTyrPheArgPheGlnGluIleLysGluAsnThrLysAsnAspLysGlnMetValGlnTyrIle  
721 TACAAATACACAAAGTTATCCTGACCCCTATATTGTTGATGAAAAAGTGCTAGAAATAGTTGTTGGTCTAAAGATGCAGAATATGGACTCTAT  
211 TyrLysTyrThrSerTyrProAspProIleLeuLeuMetLysSerAlaArgAsnSerCysTrpSerLysAspAlaGluTyrGlyLeuTyr  
811 TCCATCTATCAAGGGGAATATTTGAGCTTAAGGAAATGACAGAAATTTTGTGTTCTGTAAACAAATGAGCACCTTGATAGACATGGACCAT  
241 SerIleTyrGlnGlyIlePheGluLeuLysGluAsnAspArgIlePheValSerValThrAsnGluHisLeuIleAspMetAspHis  
901 GAAGCCAGTTTTTTCGGGGCCTTTTGTAGTTGGCTAACTGACCTGGAAAGAAAAGCAATAACCTCAAAGTGACTATTTCAGTTTTTCAGGAT  
271 GluAlaSerPhePheGlyAlaPheLeuValGlyStp  
991 GATACACTATGAAGATGTTTCAAAAAAATCTGACCAAAACAAACACAGAAA

FIG. 1

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**FIG. 2A**

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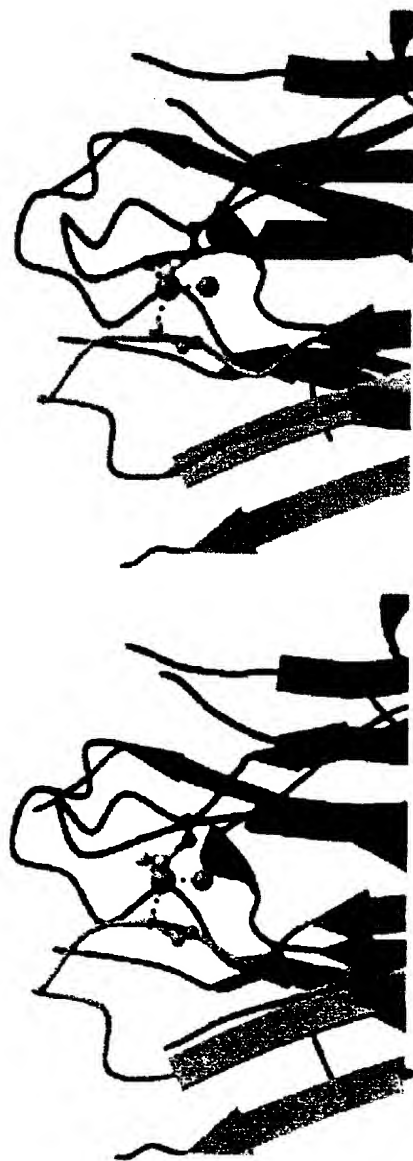
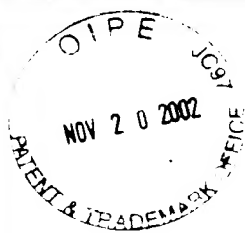


FIG.\_2B



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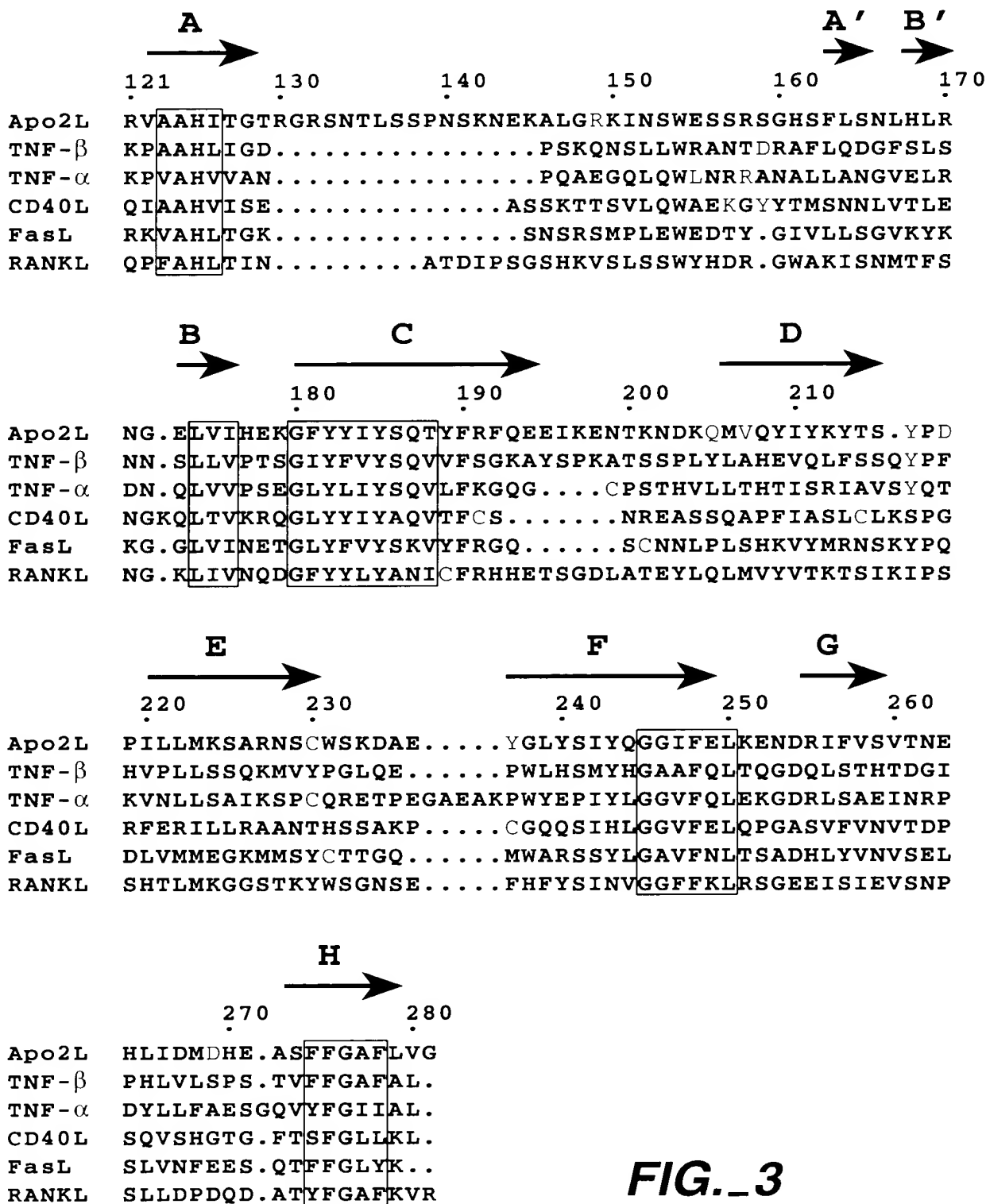
### Crystallographic Data

	<u>Apo-2L (114-281)</u>	<u>Apo-2L (91-281) D218A</u>	<u>Apo-2L (91-281) D218A</u>
Crystal			
Space Group	P6 <sub>3</sub>	R32	R32
Unit Cell (Å)	a=72.5 c=140	a=66.4 c=197.6	a=66.4 c=197.7
Resolution (Å)	3.9	1.9	1.3
Coverage (%)	94 (96)	93 (99)	100 (100)
<I/σ(I)>	5.9	10.1	12.4
# Unique (hkl)	3589	12680	41840
Redundancy	4.9	4.3	12.1
R <sub>symm</sub> (%)	15.4 (34)	6.2 (27)	6.4 (34)
# Protomers in ASU	2	1	1
Refinement			
R <sub>cryst</sub> (%)	33.8	20	
R <sub>free</sub> (%)	27.6	22	
rmsd Bonds (Å)	0.009	0.015	0.007
rmsd Angles (°)	1.79	2.0	1.41
Average B-Values	—	14	14
# Water Molecules	0	170	

$R_{\text{symm}} = \sum_h \sum_i (I_{hi} - \langle I_h \rangle) / \sum_h I$  where  $I_h$  is the mean structure factor intensity of  $i$  observations of symmetry-related reflections with Bragg index  $h$ .  $R_{\text{cryst}} = \sum_h \sum_i |F_{\text{obs}} - F_{\text{calc}}| / \sum_i |F_{\text{obs}}|$  where  $F_{\text{obs}}$  and  $F_{\text{calc}}$  are the observed and calculated structure factor amplitudes.  $R_{\text{free}} = \sum_{(hkl) \in \tau} |F_{\text{obs}(hkl)} - k F_{(hkl)}| / \sum_{(hkl) \in \tau} |F_{\text{obs}(hkl)}|$  where the  $\tau$  set of reflections is omitted from the refinement process. 10% of the data were included in the  $\tau$  set for calculation of  $R_{\text{free}}$  and not included in refinement. Values in parenthesis are for the highest resolution shell.

**FIG. 2C**

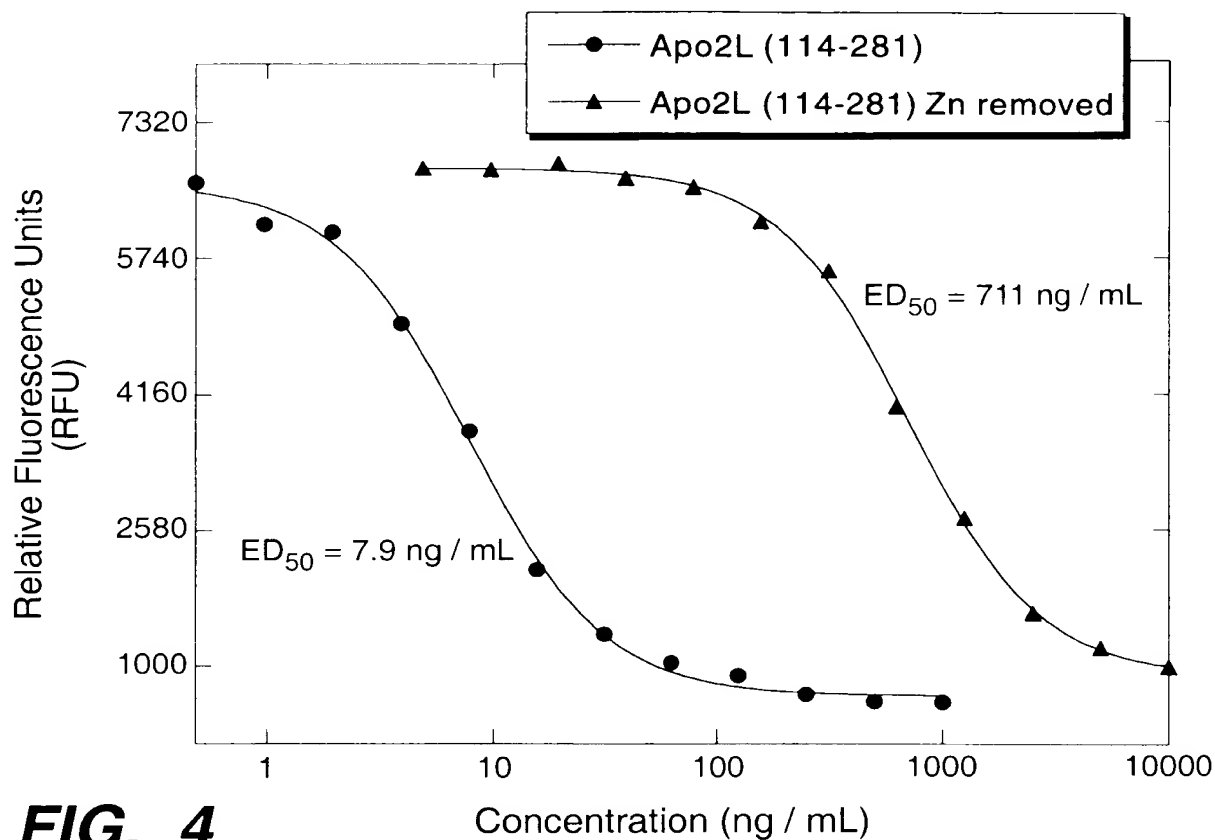
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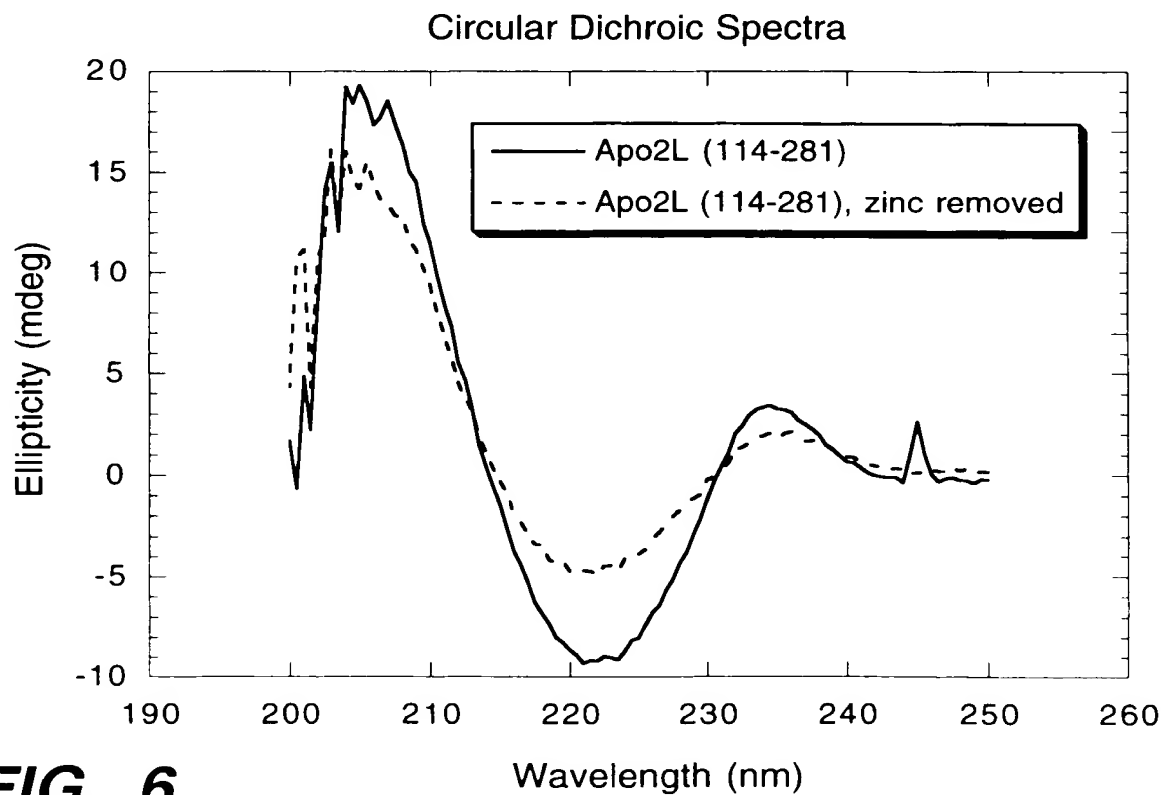
**FIG. 3**



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**FIG.\_4**



**FIG.\_6**

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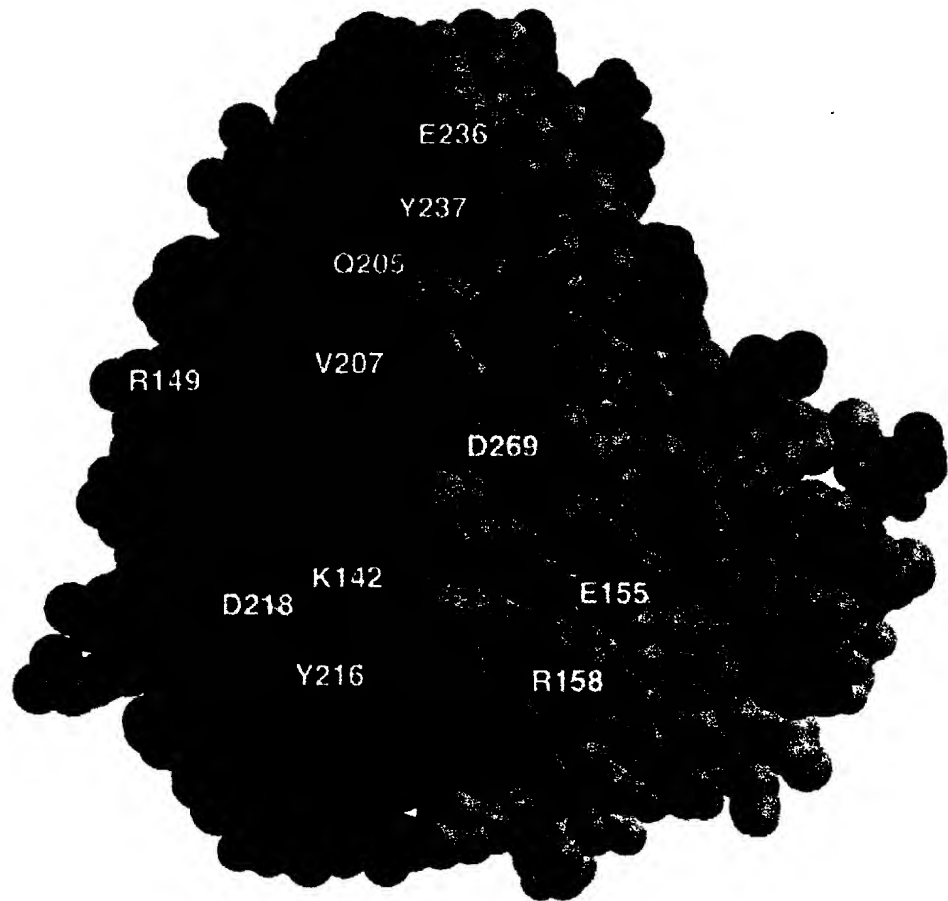
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Methods for Making Apo-2 Ligand Using Divalent Cations

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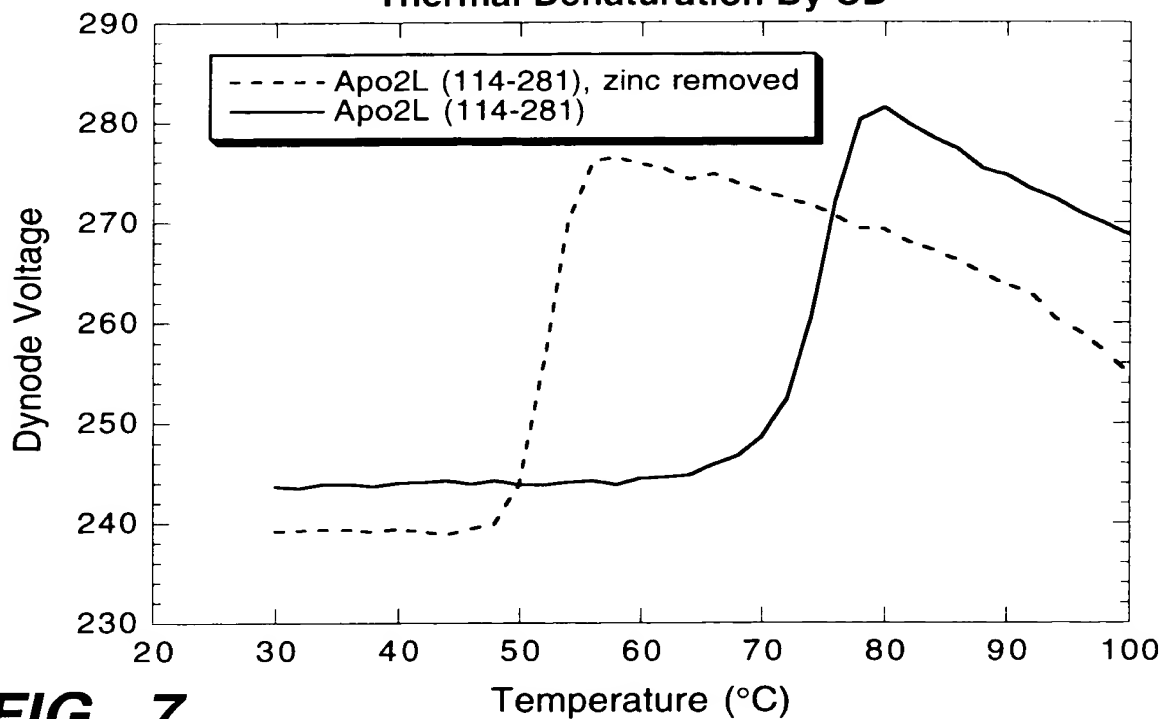


**FIG. 5**



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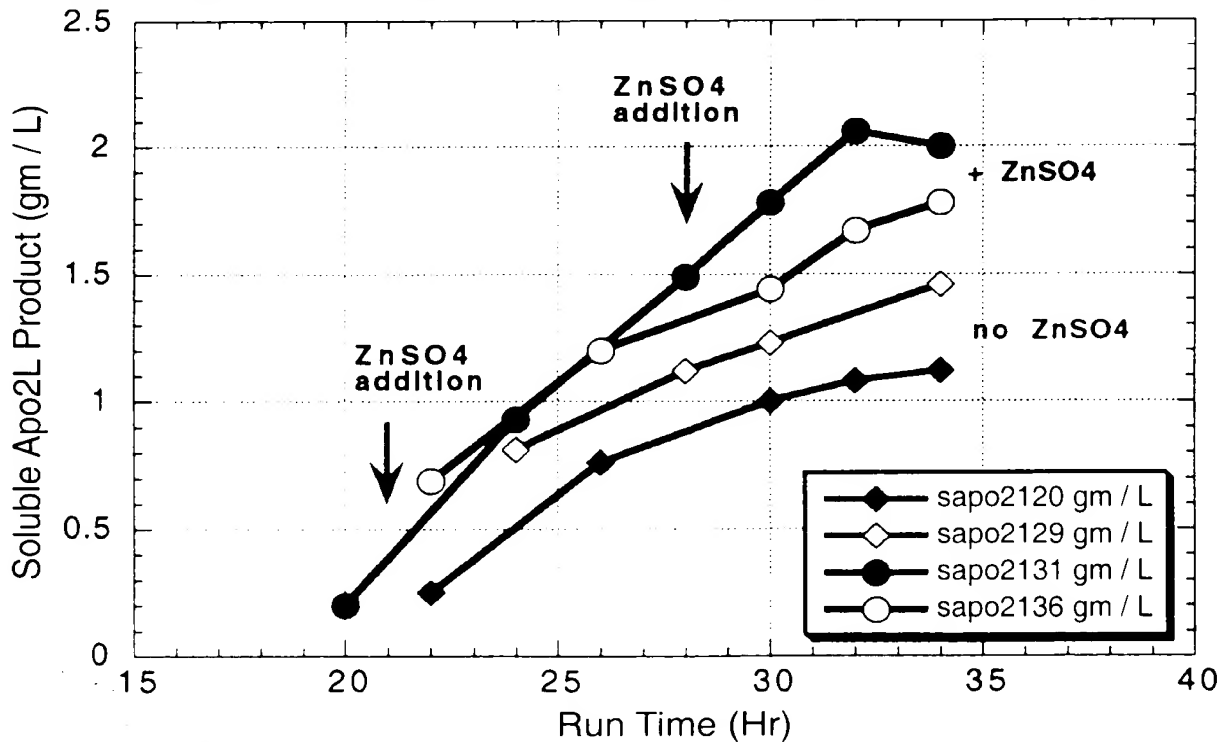
### Effect of Zn Removal On Stability: Thermal Denaturation By CD



**FIG.\_7**

### Effect of ZnSO<sub>4</sub> Additions On Apo2L Product Accumulation

Production Host: 43E7  
(E. coli W3110 fhuA (tonA) phoA Δ(argF-lac) degP kanS ptr3 ompT llvG+)



**FIG.\_8**

AP-Apo2L +/- ZnSO<sub>4</sub> gm / L



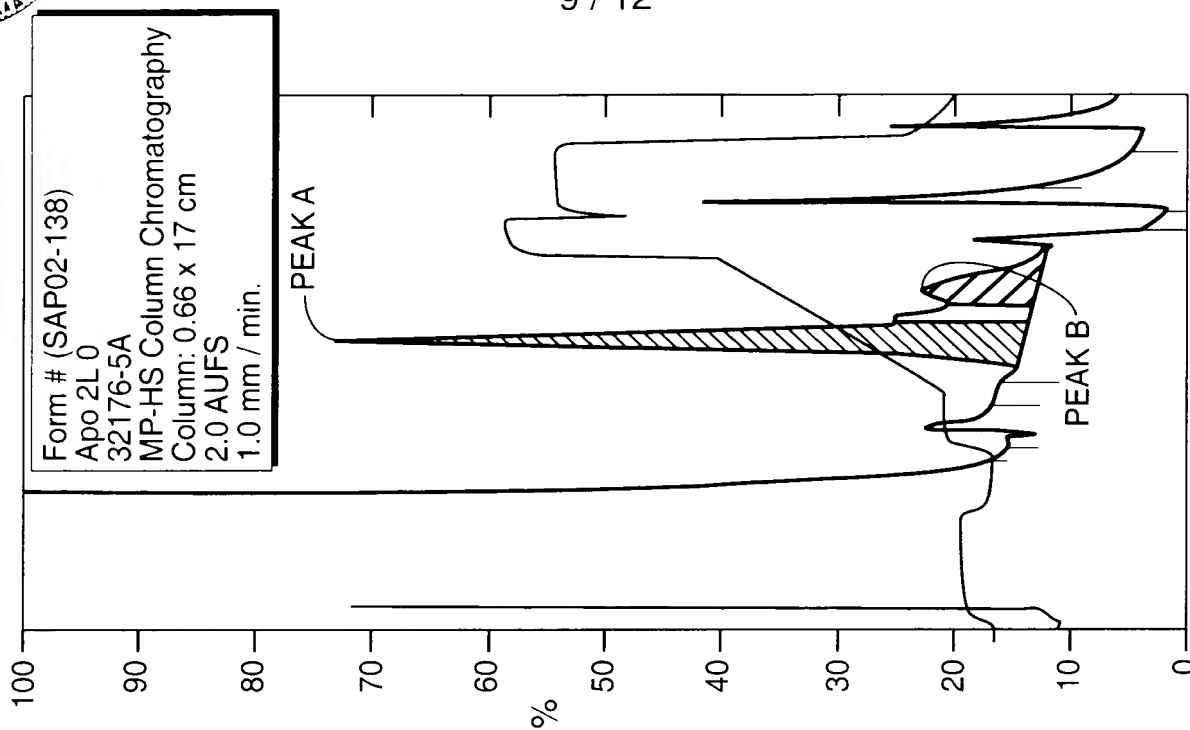
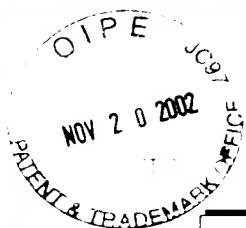


FIG. 9B

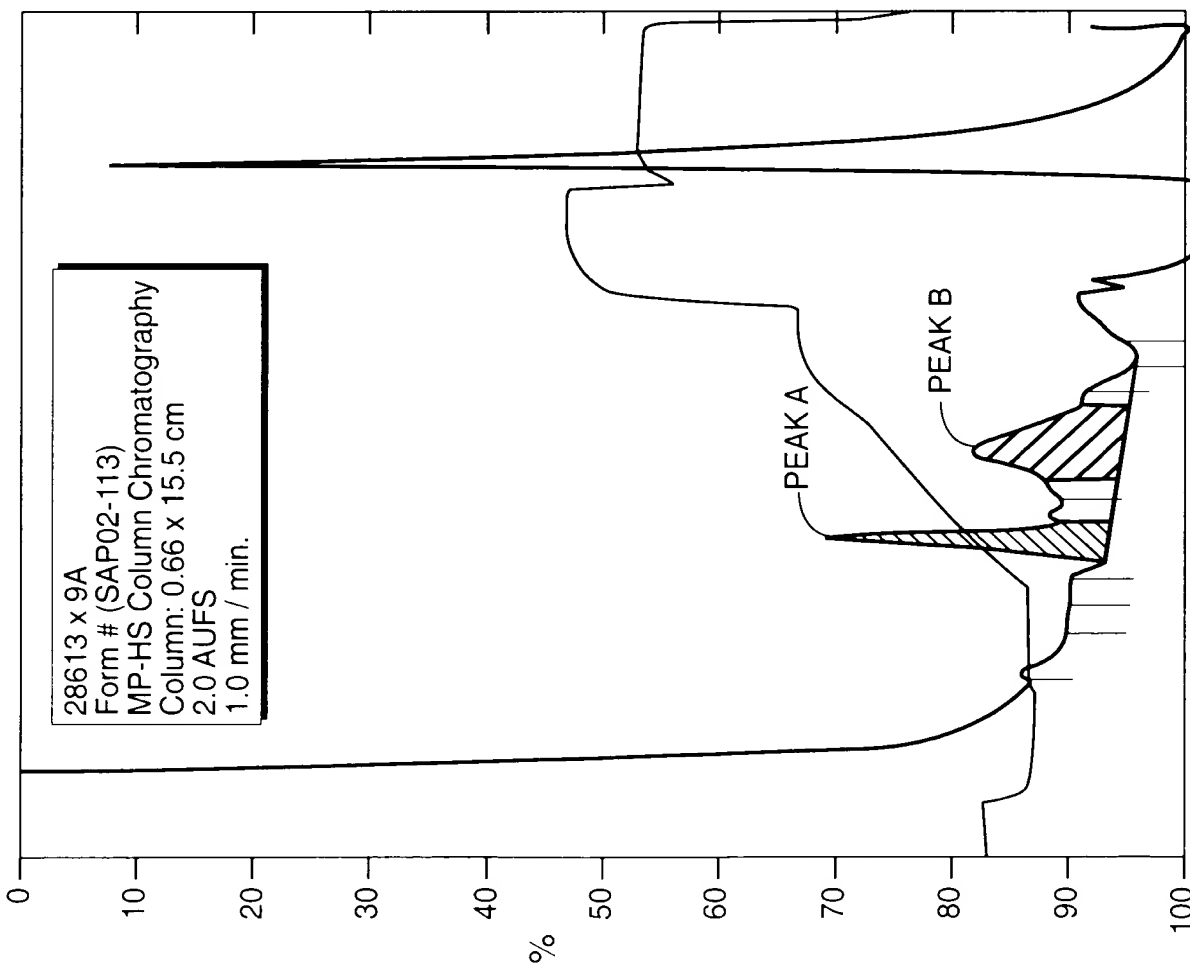
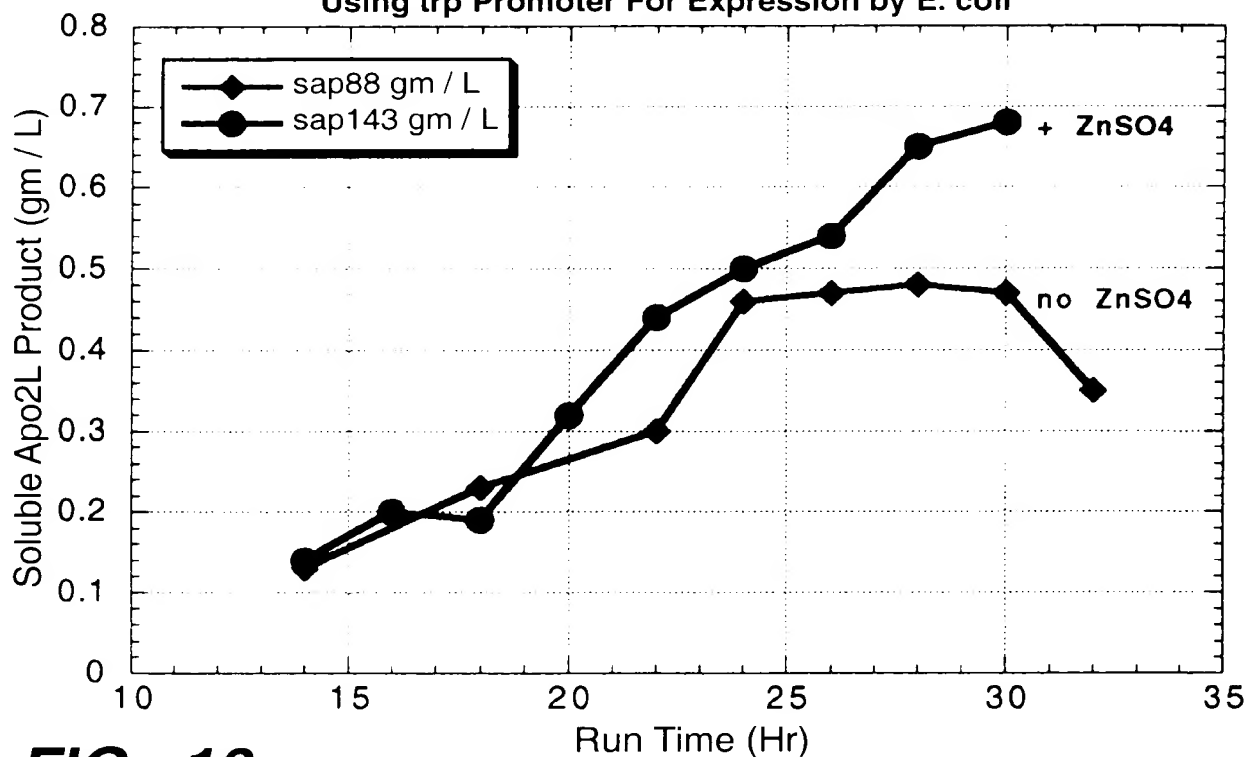


FIG. 9A

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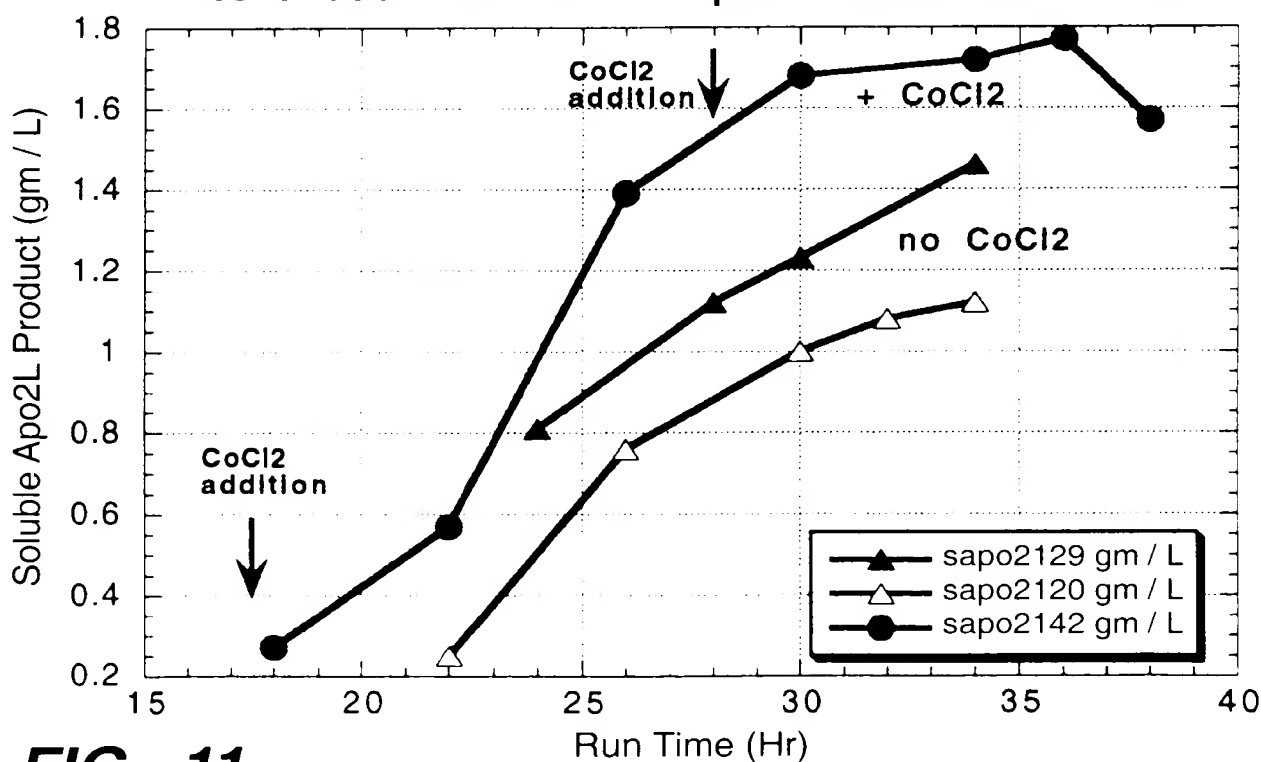
### Effect of ZnSO<sub>4</sub> Additions On Apo2L Product Accumulation Using trp Promoter For Expression by E. coli



**FIG. 10**

trp-Apo2L +/- ZnSO<sub>4</sub> gm / L

### Effect of CoCl<sub>2</sub> Additions On Apo2L Product Accumulation

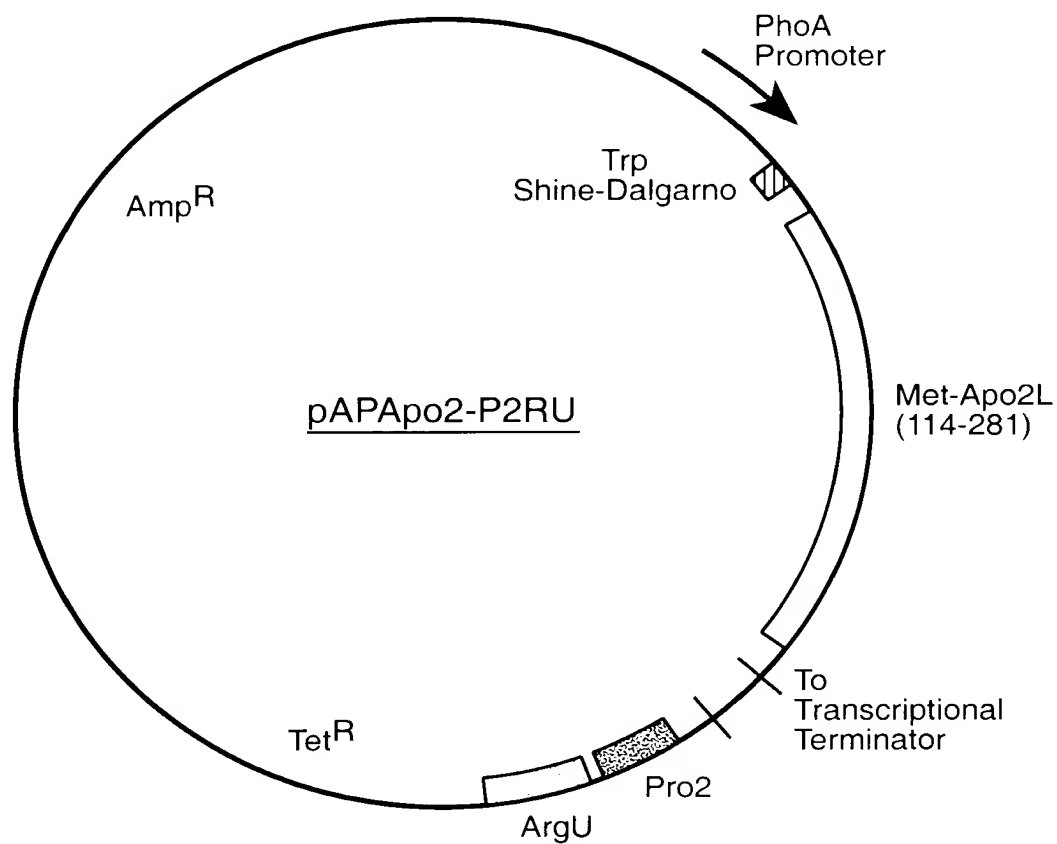


**FIG. 11**

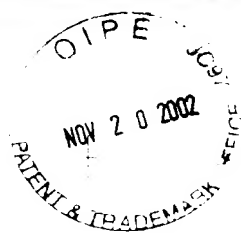
AP-Apo2L + CoCl<sub>2</sub> gm / L



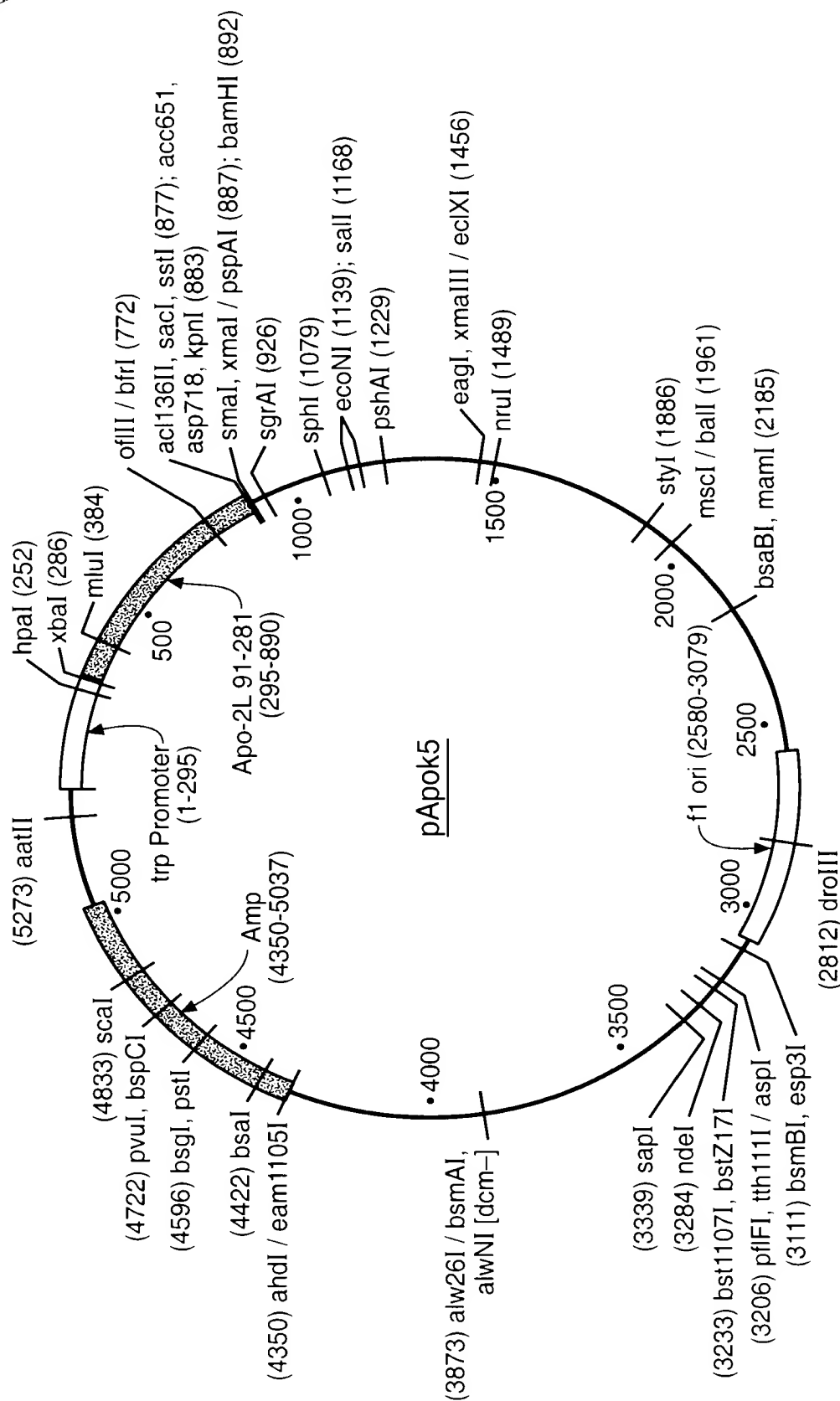
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**FIG. 12**



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**FIG. 13**